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Genetic structure of *Picea abies* populations growing on extreme sites as revealed by isoenzyme markers: a case study from Slovenia and Bosnia and Herzegovina

Abstract: Three populations of Norway spruce from ecologically extreme environments in Slovenia and Bosnia and Herzegovina were examined for genetic polymorphism. The spruces there grow in specific forest communities (*Sphagno-Piceetum*) which represent the remnants of the post-glacial vegetation. The aim of the study was to search for similarities in the genetic variation among populations adapted to such conditions. In total, 10 isoenzyme systems involving 16 gene loci were analysed. The results showed differences in genetic differentiation at loci *Got-B*, *Skdh-A* and *6-Pgdh-C* between the two Slovenian populations and the Bosnian population, but also indicated an interestingly close relationship between the Slovenian population Pohorje and the Bosnian population Nišići.

Additional key words: Norway spruce, genetic variation, genetic polymorphism

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Introduction

Norway spruce (*Picea abies* (L.) Karst.), one of the most valuable forest tree species, plays a significant economic and ecological role in Slovenia, Bosnia and Herzegovina, and many other European countries (Skroppa 2003). Analyses of the isoenzyme loci polymorphism of Norway spruce revealed a high level of genetic variation in Europe, and a wide differentiation between the populations (Müller-Starck et al. 1992). Other studies also reported a high level of genetic variation and population differentiation in this species (Goncharenko et al. 1990; Goncharenko and Potenko 1992; Krutovskii and Bergmann 1995). Langercrantz and Ryman (1990) consider that the species is in the continuous process of genetic differentiation and adaptation under varied ecological conditions.

Earlier studies confirmed that Norway spruce responds well to various selection pressures and ecological factors, which may be traced in its population genetic structure. The differences in genetic structure between populations are particularly attributed to different site factors such as soil parameters (Božič 2002; Božič and Urbančič 2001, 2003) or climate (Ballian et al. 2006), and to many atmospheric and soil pollutants (Bergmann and Scholz 1987, 1989; Hosius and Bergmann 1993; Bergmann and Hosius 1996; Longauer et al. 2001).

The objective of this study was to examine the level of genetic relatedness among forest communities of spruce on turf by applying biochemical molecular genetic methods and analysing their results. We looked at slowly-growing autochthonous spruce populations in Slovenia and Bosnia and Herzegovina, which are

adapted to extremely poor and severe site conditions. We wanted to find out whether the respective provenances have the expected genetic structures, and to establish the extent of genetic differentiation among them. As the geographic distance between the populations from Bosnia and those from Slovenia is considerable, we expected some differences in allele frequencies. It was assumed that natural selection pressures, which influence these populations, may shape their genetic structures in similar ways.

Material and methods

Description of populations

Jelovica is a high mountain plateau in the Julian Alps, situated at 1000–1200 m a.s.l. (Table 1). The entire area of Jelovica is influenced by the alpine continental humid climate. The annual precipitation in the area ranges from 2000 to 3000 mm, and during the vegetation period it is between 1000 and 1500 mm. In this region, spruce naturally grows in edaphogenous communities *Sphagno-Piceetum* in a marshy area, while *Rhytidiadelpho lorei-Piceetum* s. lat. grows in the surroundings of a marshy land where the environment is somewhat drier (Zupančič 1999).

Pohorje is a flattened mountain massif with an altitude between 1200 and 1500 m, forming a part of the Central Alps (Table 1). It is built of metamorphous and magma sediments (Budnar-Tregubov 1958). The annual precipitation in the area is 1500 mm, with 800–900 mm in the vegetation period (Zupančič 1999). The climate is alpine, humid, with Pannonian influences. The forests covering a major part of the Pohorje plateau have a clear and concurrent spruce structure. At aslope and slightly better quality soils, minor groups of autochthonous clusters/stands have been preserved that include other tree species. At high plateaus and peaks of the central parts of Pohorje, spruce has also endured as a permanent stand despite extreme climatic conditions (strong winds, frost, and short vegetation period), as well as unfavourable conditions of soil (marshy grounds).

Nišička visoravan (Nišiči plateau) is a mountain plateau with the altered continental climate (Table 1). The spruce forest here is influenced by orographic and edaphic factors. It represents a permanent stage of vegetation on flat lands where the soil is pseudogley with transition from marshy and turf soils. Apart from the increased soil humidity and local climatic

conditions (frost), the altitudes around 1000 m also make spruce a sole edifier. The climate is alpine, with an average annual temperature of 7.1°C and annual precipitation of 1180 mm, with 567 mm in the vegetation period. Because of that, the forest community there, i.e. *Sphagno-Piceetum montanum* Stef. 1964, has a limited distribution. It was first reported from a complex of verphene sediments which are widely distributed southeast, north and northeast of Sarajevo (Nišiči–Olovo–Vareš) (Stefanović 1977; Stefanović et al. 1983).

Sampling

During January 2005, we sampled branches with dormant buds. During the collection of samples for isoenzymatic studies, we ensured that the distance between individuals is at least 50 to 100 m (in order to avoid inbreeding/crossbreeding), and that the trees are older than 80 years. In each population, 50 individuals were sampled.

Isoenzymatic analysis

Genetic structure was analysed using isoenzyme loci *Idh-A*, *Idh-B*, *Mdh-A*, *Mdh-B*, *Mdh-C*, *Lap-B*, *Got-A*, *Got-B*, *Got-C*, *Pgm-A*, *Pgi-B*, *Gdh-A*, *Skdh-A*, *6-Pgdh-B*, *6-Pgdh-C*, and *F-Est-B*. The zymogram was interpreted according to the protocol given by Konnert (2004). Genetic variation was evaluated by the measures of genetic variability for polymorphic loci in at least one of the population. On the basis of allele and genotype frequencies, genetic variability within populations was quantified by measures of genetic diversity and heterozygosity: n – effective number of alleles per locus, n_{gam} – hypothetical gametic multilocus diversity (Gregorius 1978), H_a – actual, observed level of heterozygosity, d_T – intra-population differentiation. Genetic variability between the sampled populations was studied by statistical testing of the homogeneity of allele distributions using Fisher's exact test, and by measures of genetic differentiation: d_0 – genetic distance, D_j – subpopulation genetic differentiation. Genetic parameters were calculated using programs GSED version 1.1e (Gillet 1998) and GENEPOP version 3.1d.

Results

Allele and genotype frequencies for the three spruce populations at 16 analysed loci are listed in Tables 2 and 3.

Table 1. Site characteristics of Norway spruce populations studied in Slovenia (SLO) and Bosnia and Herzegovina (BiH)

Population	Locality	Latitude	Longitude	Alt. (m)	Forest community	Country
Bijambare	Nišička visoravan	44°05'01''	18°29'39''	950	<i>Sphagno-Piceetum montanum</i>	BiH
Blatni greben	Jelovica	46°17'07''	14°04'31''	1050	<i>Sphagno-Piceetum</i>	SLO
Trije kralji	Pohorje	46°28'08''	15°24'32''	1300	<i>Sphagno-Piceetum</i>	SLO

Table 3. Genotype frequencies for 16 gene loci in sampled Norway spruce populations

No.	Locus	Genotype	Genotype frequency			No.	Locus	Genotype	Genotype frequency				
			Jelovica	Pohorje	Nišiči				Jelovica	Pohorje	Nišiči		
1	<i>Gdh-A</i>	22	1.000	1.000	1.000	9	<i>Mdh-B</i>	12	0.040	–	–		
								22	0.940	1.000	1.000		
2	<i>Got-A</i>	12	0.040	–	–			23	0.020	–	–		
		22	0.960	1.000	1.000								
3	<i>Got-B</i>	12	–	–	0.040	10	<i>Mdh-C</i>	24	0.060	0.020	0.060		
		22	1.000	1.000	0.900			44	0.940	0.980	0.940		
		23	–	–	0.060								
4	<i>Got-C</i>	22	0.300	0.100	0.120	11	<i>Pgi-B</i>	22	0.100	0.060	0.140		
		24	0.460	0.440	0.460			23	0.400	0.400	0.300		
		25	–	0.040	0.020			33	0.500	0.520	0.560		
		44	0.240	0.380	0.380			34	–	0.020	–		
		45	–	0.040	0.020								
5	<i>Idh-A</i>	13	–	–	0.060	12	<i>Pgm-A</i>	22	0.820	0.780	0.860		
		22	–	–	0.020			23	0.160	0.200	0.120		
		23	0.020	0.080	0.080			24	0.020	0.020	–		
		33	0.980	0.920	0.820			33	–	–	0.020		
		34	–	–	0.020								
6	<i>Idh-B</i>	11	–	–	0.020	13	<i>Skdh-A</i>	13	0.020	–	0.080		
		33	1.000	1.000	0.980			23	0.060	0.020	0.100		
7	<i>Lap-B</i>	23	0.040	–	–	14	<i>6-Pgdh-B</i>	12	–	–	0.020		
		24	–	0.020	–			15	0.020	–	–		
		33	–	–	0.040			22	0.420	0.420	0.360		
		34	0.220	0.200	0.260			23	–	–	0.080		
		44	0.520	0.660	0.660			25	0.500	0.460	0.440		
		45	–	0.020	–			55	0.060	0.120	0.100		
		46	0.140	0.040	0.040			15	<i>6-Pgdh-C</i>	22	0.340	0.340	0.480
		47	–	0.040	–					25	0.400	0.360	0.420
		66	0.080	0.020	–					55	0.260	0.300	0.100
8	<i>Mdh-A</i>	22	1.000	1.000	1.000	16	<i>F-Est-B</i>	11	–	0.020	–		
								12	0.060	0.120	0.060		
								22	0.900	0.860	0.088		
								24	0.040	–	0.060		

common in Jelovica and Pohorje than in Nišiči (26–30% vs. 10%). The Slovenian provenance Jelovica differs greatly from the other Slovenian provenance Pohorje and the Bosnian provenance Nišiči at loci *Got-C*, *Idh-A* and *Lap-B*. For example, Jelovica has a higher frequency of allele *Got-C*₂ (53%) than either Pohorje or Nišiči (34–36%), and a lower frequency of allele *Got-C*₄ (47%) compared to the other two populations (62%). At this gene locus, homozygote *C*₂₂ is much more frequent in Jelovica (30%) than in Pohorje and Nišiči (10 and 12%). At locus *Lap-B*, too,

the frequency of allele *B*₆ is much higher in Jelovica (15%) than in Pohorje and Nišiči (up to 4%). Locus *Idh-A* is close to fixation only in the population of Jelovica where allele *A*₃ reaches a frequency of 99%.

Genetic variation within populations

The intra-population variation in the three spruce populations was examined using different measures of genetic diversity and heterozygosity (Tables 4 and 5).

The number of alleles identified in the populations varies from 32 (Pohorje, Slovenia) to 36 (Nišiči,

Bosnia and Herzegovina); the latter population has also the highest number of private alleles. The genetic diversity (n) ranges between 1.233 (Pohorje) and 1.275 (Nišići; Table 4). The hypothetical gametic multilocus diversity (n_{gam}) also tends to be highest in Nišići. Compared to the Pohorje population, the populations of Jelovica and Nišići have a greater diversity. The differences in actual (observed) heterozygosity between the three populations are negligible (18.3, 20, 6%). The genetic differentiation (d_T) which, due to the method of computation can be expected to be similar to the expected heterozygosity, lies between 19.1% (Pohorje) and 21.8% (Nišići; Table 4). A comparison between the observed heterozygosity values (H_a) and the differentiation values (d_T , %) shows a nonsignificant or scarce number of heterozygotes in the population growing under extreme conditions.

In all populations, the loci that exhibited highest heterozygosity are as follows: *6-Pgdh-B* (46–54%), *Got-C* (46–52%), *6-Pgdh-C* (36–42%), *Pgi-B* (30–42%), and *Lap-B* (30–40%), and those with lowest heterozygosity are *Idh-B*, *Mdh-B* and *Got-A* (0–10%; Table 5).

Table 4. Average number of alleles (A/L), effective number of alleles per locus (n), hypothetical gametic multilocus diversity (n_{gam}), actual multilocus heterozygosity (H_a , %), and intra-population genetic differentiation (d_T , %) of sampled Norway spruce populations for 14 polymorphic gene loci

Population	A/L	n	n_{gam}	H_a	d_T
Jelovica	2.312	1.260	41.942	19.9	20.8
Pohorje	2.062	1.233	29.873	23.9	19.1
Nišići	2.375	1.275	43.848	21.7	21.8

Table 5. Actual heterozygosity (H_a , %) of sampled Norway spruce populations for 14 polymorphic gene loci

Locus	H_a		
	Jelovica	Pohorje	Nišići
<i>Idh-A</i>	2.0	8.0	16.0
<i>Idh-B</i>	0	0	0
<i>Mdh-B</i>	6.0	0	0
<i>Mdh-C</i>	6.0	2.0	6.0
<i>Lap-B</i>	40.0	32.0	30.0
<i>Got-A</i>	4.0	0	0
<i>Got-B</i>	0	0	10.0
<i>Got-C</i>	48.9	68.4	65.8
<i>Pgm-A</i>	18.0	22.0	12.0
<i>Pgi-B</i>	40.0	42.0	30.0
<i>Skdh-A</i>	12.0	4.0	26.0
<i>6-Pgdh-B</i>	52.0	46.0	54.0
<i>6-Pgdh-C</i>	40.0	36.0	42.0
<i>Fest-B</i>	10.0	75.0	12.0
Average	19.9	23.9	21.7

Genetic variation among populations

The Fisher exact test for homogeneity of the allelic frequencies of spruce populations was performed for the 14 gene loci analysed. The P -values indicated significant deviations from homogeneity of the allelic frequencies. By the Fisher exact test we also verified that there is a statistically significant difference for 5 out of 14 polymorphic gene loci (Table 6).

Looking at the data provided in Tables 2, 3 and 6 for loci *Got-B* and *Skdh-A* we can conclude that major differences exist between the Slovenian populations on the one hand and the Bosnian population on the other. For loci *Got-C*, *Idh-A* and *Lap-B*, however, it is interesting that the populations of Pohorje and Nišići only little contribute to the statistically significant difference obtained, but major differences exist between the populations of Jelovica and Nišići.

The allelic and genotypic gene pool distances between the populations range, respectively, from 4.5% and 7.0% (between the Slovenian provenance Jelovica and Pohorje) to 5.9% and 10.1% (between the Slovenian population Jelovica and the Bosnian population Nišići; Table 7). All the values are slightly higher for the distances between the Slovenian population Pohorje and the Bosnian population Nišići than for those between the two Slovenian populations which are genetically closer to each other. The reasons could be traced to the greater similarity of the allele frequencies for some gene loci, as well as the genotype frequency.

Based on the maximum and minimum allelic genetic distances (d_0) between the populations for the gene loci analysed, it can be found that the minimum values of d_0 between the Pohorje and Nišići populations occur at loci *Mdh-B*, *Lap-B*, *Got-A* and *Got-C*, and the maximum values occur at loci *Idh-B*, *Mdh-C*, *Got-B*, *Skdh-A*, *Pgm-A*, *6Pgdh-B* and *6Pgdh-C* (Table 8).

As suggested by the results shown in Table 8, the population Pohorje is closer than Jelovica to the population Nišići, which corroborates the results from Table 7.

The range of genetic differentiation (D_j) among the spruce populations for the loci analysed is presented in Table 9. The difference between the populations in D_j terms is widest at gene locus *Got-C* (11.5%), fol-

Table 6. Fisher exact test for allele frequencies

Locus	P -value	Locus	P -value
<i>Idh-A</i>	13.24*	<i>Got-C</i>	13.38**
<i>Idh-B</i>	4.42 ^{ns}	<i>Pgm-A</i>	1.97 ^{ns}
<i>Mdh-B</i>	6.65 ^{ns}	<i>Pgi-B</i>	2.57 ^{ns}
<i>Mdh-C</i>	1.34 ^{ns}	<i>Skdh-A</i>	17.16*
<i>Lap-B</i>	25.75**	<i>6Pgdh-B</i>	10.71 ^{ns}
<i>Got-A</i>	4.42 ^{ns}	<i>6Pgdh-C</i>	7.23 ^{ns}
<i>Got-B</i>	11.15*	<i>Fest-B</i>	7.62 ^{ns}

* $p < 0.05$, ** $p < 0.01$, ns – nonsignificant ($p \geq 0.05$)

Table 7. Allelic and genotypic genetic distances (d_0 , %) between sampled Norway spruce populations for 14 polymorphic loci and for gene pool

Locus	Allelic distance			Genotypic distance		
	POP1 / POP2	POP1 / POP3	POP2 / POP3	POP1 / POP2	POP1 / POP3	POP2 / POP3
<i>Idh-A</i>	3.0	9.0	6.0	6.0	16.0	10.0
<i>Idh-B</i>	0	2.0	2.0	0	2.0	2.0
<i>Mdh-B</i>	3.0	3.0	0	6.0	6.0	0
<i>Mdh-C</i>	2.0	0	2.0	4.0	0	4.0
<i>Lap-B</i>	15.0	15.0	7.0	22.0	22.0	10.0
<i>Got-A</i>	2.0	2.0	0	4.0	4.0	0
<i>Got-B</i>	0	5.0	5.0	0	10.0	10.0
<i>Got-C</i>	19.0	17.0	2.0	22.0	18.0	4.0
<i>Pgm-A</i>	2.0	1.0	3.0	4.0	6.0	10.0
<i>Pgi-B</i>	4.0	1.0	3.0	4.0	10.0	12.0
<i>Skdh-A</i>	4.0	8.0	12.0	8.0	16.0	24.0
<i>6-Pgdh-B</i>	3.0	4.0	5.0	6.0	14.0	10.0
<i>6-Pgdh-C</i>	2.0	15.0	17.0	4.0	16.0	20.0
<i>Fest-B</i>	5.0	1.0	5.0	8.0	2.0	8.0
<i>Gene pool</i>	4.5	5.9	4.9	7.0	10.1	8.8

POP1 – Jelovica, POP2 – Pohorje, POP3 – Nišiči

Table 8. Minimum and maximum allelic genetic distances (d_0)

Locus	Min d_0	Relationship	Max d_0	Relationship
<i>Idh-A</i>	0.030	POP 1–2	0.090	POP 1–3
<i>Idh-B</i>	0	POP 1–2	0.020	POP 1–3 / 2–3
<i>Mdh-B</i>	0	POP 2–3	0.030	POP 1–2 / 1–3
<i>Mdh-C</i>	0	POP 1–3	0.020	POP 1–2 / 2–3
<i>Lap-B</i>	0.070	POP 2–3	0.150	POP 1–2 / 1–3
<i>Got-A</i>	0	POP 2–3	0.020	POP 1–2 / 1–3
<i>Got-B</i>	0	POP 1–2	0.050	POP 1–3 / 2–3
<i>Got-C</i>	0.020	POP 2–3	0.190	POP 1–2
<i>Pgm-A</i>	0.010	POP 1–3	0.030	POP 2–3
<i>Pgi-B</i>	0.010	POP 1–3	0.040	POP 1–2
<i>Skdh-A</i>	0.040	POP 1–2	0.120	POP 2–3
<i>6-Pgdh-B</i>	0.030	POP 1–2	0.050	POP 2–3
<i>6-Pgdh-C</i>	0.020	POP 1–2	0.170	POP 2–3
<i>Fest-B</i>	0.010	POP 1–3	0.050	POP 1–3 / 2–3

POP1 – Jelovica, POP2 – Pohorje, POP3 – Nišiči

lowed by *6Pgdh-C* (9.5%). These findings confirm previous reports which pointed to the existence of diverse selection pressures that govern those populations and influence their genetic structure.

Discussion

We compared the genetic structures of autochthonous spruce populations exhibiting slow and dwarf-like growth, which typically appear at extreme *Sphagno-Piceetum* sites in Slovenia and Bosnia and Herzegovina. All the three populations have a wide ecological valence and are well adapted to their re-

spective environments due to the long evolution under the influence of natural selection. Comparison of their genetic structures allows us to better understand these adaptation paths.

The populations show marked differences in allelic and genotypic structures, with significant differences in allelic frequencies. At loci *Got-B*, *Skdh-A* and *6-Pgdh-C*, both Slovenian populations differ from the Bosnian population. At loci *Got-C*, *Idh-A* and *Lap-B*, the Slovenian population Jelovica substantially differs from the other Slovenian population Pohorje and the Bosnian population Nišiči. All the three populations exhibit also a high level of genetic variability within

Table 9. Genetic differentiation (D_3) of sampled Norway spruce populations by loci, presented with average gene loci values (d_3), and average differentiation of population gene pool, in %

Locus	POP1	POP2	POP3	d_3
<i>Idh-A</i>	0.060	0.020	0.075	0.052
<i>Idh-B</i>	0.010	0.010	0.020	0.013
<i>Mdh-B</i>	0.030	0.015	0.015	0.020
<i>Mdh-C</i>	0.010	0.020	0.010	0.013
<i>Lap-B</i>	0.135	0.095	0.105	0.112
<i>Got-A</i>	0.020	0.010	0.010	0.013
<i>Got-B</i>	0.025	0.025	0.050	0.033
<i>Got-C</i>	0.180	0.105	0.075	0.120
<i>Pgm-A</i>	0.010	0.025	0.020	0.018
<i>Pgi-B</i>	0.025	0.035	0.010	0.023
<i>Skdh-A</i>	0.020	0.080	0.100	0.067
<i>6-Pgdh-B</i>	0.035	0.030	0.045	0.037
<i>6-Pgdh-C</i>	0.065	0.095	0.160	0.107
<i>Fest-B</i>	0.025	0.050	0.025	0.033
<i>Gene pool</i>	0.046	0.044	0.051	0.047

POP1 – Jelovica, POP2 – Pohorje, POP3 – Nišiči

populations. Some of the alleles are rare and show particular characteristics only for an equivalent (matching/reciprocal) population; there is also heterozygosity of some alleles. This is the significance of the Nišiči (*Got-B*, *Idh-A*) and Jelovica (*Got-A*, *Mdh-B*) populations with two specific gene loci each. Genetic variability within populations, as well as between the populations, exhibits a similar pattern to heterozygosity where an individual gene locus contributes considerably more to variability than others. The high heterozygosity of loci *Lap-B*, *Got-C*, *Pgi-B*, *6Pgdh-B*, *C* and *6Pgdh-C* indicates that selection processes had a positive effect on these loci. In the case of other loci, such an effect is much less pronounced or totally missing. For the population of Nišiči we can thereby confirm that there is possibly a selection pressure that favours a high heterozygosity of locus *Skdh-A*, while at three loci there is monomorphism and this type of selection does not occur. Heterozygosity obtained in this way suggests that the three subpopulations are subject to diverse selection pressures. The differences between the populations correspond with their geographic distances, but the differences in climate might also be significant and affect the genetic structure.

The intra-population variability is highest in the population of Nišiči, possibly due to its character as a relict area and the proximity of the Balkan glacial refugium, and lowest in the Pohorje population; this may have resulted from the population proportions as well as the influence of different selection pressures.

Heterozygosity shows the same pattern as diversity, the differences between the populations, however, are nonsignificant.

The genetic distances (d_0) are equal to those found by Božič (2002) between spruce from turf and typical stands in Pokljuka (the Jelovica population) in Slovenia, as well as by Ballian et al. (2007) in subpopulations on the mountain of Igman. As expected, the two populations from Slovenia (Jelovica and Pohorje) show a high level of relatedness. It is noteworthy, however, that the Bosnian population (Nišiči) displays a greater relatedness to the population of Pohorje than to that of Jelovica.

The reason for smaller differences between Pohorje and Nišiči may be seen in the effects of similar specific selection pressures produced by environmental factors. Such a conclusion is supported by the results of the allele and genotype analysis, the data on heterozygosity, and the analysis of allelic and genotypic distances. The minimum allelic genetic distances between the Jelovica and Pohorje populations occur in 6 cases, between the Jelovica and Nišiči populations in 3 cases, and between the Pohorje and Nišiči populations in 4 cases. By contrast, the maximum distances between Pohorje and Nišiči exist in 8 cases, between Jelovica and Pohorje in 6 cases, and between Jelovica and Nišiči in 6 cases. Many previous studies pointed to the vertical differentiation of spruce (Müller-Starck 1989; Krajmerová and Longauer 2000; Pacalaj et al. 2002; Ballian et al. 2007). If we take account of altitude and consider the vertical stratification of the populations studied, we will not be able to draw a valid conclusion because the position of the populations would suggest a greater closeness between the populations of Jelovica and Nišiči than Pohorje and Nišiči.

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References

- Ballian D., Bogunić F., Konnert M., Kraigher H., Pučko M., Božič G. 2007. Genetička diferenciranost subpopulacija obične smreke (*Picea abies* (L.) Karst.) na planini Igman. Šumarski list 1–2: 13–23.
- Bergmann F., Hosius B. 1996. Effects of heavy-metal polluted soils on the genetic structure of Norway spruce seedling populations. *Water, Air and Soil Pollution* 89: 363–373.
- Bergmann F., Scholz F. 1987. The impact of air pollution on the genetic structure of Norway spruce. *Silvae Genetica* 36(2): 80–83.
- Bergmann F., Scholz F. 1989. Selection effects of air pollution in Norway spruce (*Picea abies*) populations. In: *Genetic Effects of Air Pollutants in Forest Tree Populations*. Scholz F., Gregorius H.R., Rudin D. (eds.). Springer Verlag, Berlin, pp. 143–160.
- Božič G. 2002. Subpopulation differentiation under different forest site conditions within autochthonous Norway spruce (*Picea abies* (L.) Karst.) population. *Razprave IV. Razreda SAZU, XLIII-3*: 95–109, Ljubljana.
- Božič G., Urbančič M. 2001. Influences of the soils on the morphological characteristics of an autochthonous Norway spruce on the Pokljuka plateau. *Glasnik za Šumske Pokuse* 38: 137–147.
- Božič G., Urbančič M. 2003. The morphological and genetical characterisation of native Norway spruce (*Picea abies* (L.) Karst.) population in the area of Pokljuka mire. *Acta Biologica Slovenica* 46(1): 17–25.
- Budnar-Tregubov A. 1958. Palinološko raziskovanje barij na Pokljuki in Pohorju. *Geologija, Razprave in poročila, Ljubljana* 4: 197–220.
- Gillet E.M. 1998. GSED – Genetic Structure from Electrophoresis Data, Version 1.1e. Institut fuer Forstgenetik und Forstpflanzenzuechtung Universitaet Gettingen.
- Goncharenko G.G., Potenko V.V. 1992. Genetic variability and differentiation in Norway spruce (*Picea abies* (L.) Karst.) and Siberian spruce (*Picea obovata* Ledeb.) populations. *Soviet Genetics* 27: 1235–1246.
- Goncharenko G.G., Potenko V.V., Slobodyan J.N., Sidor A.I. 1990. Genetic and taxonomic relations between *Picea abies* (L.): *montana* Schur. and *P. obovata* Ledeb. *Doklady Akademii Nauk BSSR* 34: 361–364.
- Gregorius H.R. 1978. The concept of genetic diversity and differentiation. *Theoretical and Applied Genetics* 74, 397–401.
- Hosius B., Bergmann F. 1993. Adaptation of Norway spruce to heavy metal contaminated soil. In: *Norway Spruce Provenances and Breeding*. Rone V. (ed.). Latvian Forest Research Institute 'Silva', Riga, pp. 200–207.
- Konnert M. 2004. *Handbücher für Isoenzymanalyse*.
- Krajmerová D., Longauer R. 2000. Genetička diverzita smreka običajného na Slovensku. *Forestry Journal* 46(3): 273–286.
- Krutovskii V.K., Bergmann F. 1995. Introgressive hybridization and phylogenetic relationships between Norway, *Picea abies* (L.) Karst., and Siberian, *Picea obovata* Ledeb., spruce species studied by isozyme loci. *Heredity* 74: 464–480.
- Langercrantz U., Ryman N. 1990. Genetic structure of Norway spruce (*Picea abies*): concordance of morphological and allozymic variation. *Evolution* 44: 38–53.
- Longauer R., Gömöry D., Paule L., Karnosky D.F., Maňková B., Müller-Starck G., Percy K., Szaro R. 2001. Selection effects of air pollution on gene pools of Norway spruce, European silver fir and European beech. *Environmental Pollution* 115: 405–411.
- Müller-Starck G. 1989. Genetic implications of environmental stress in adult forest stands of *Fagus sylvatica* L. In: *Genetic Effects of Air Pollutants in Forest Tree Populations*. Scholz F., Gregorius H.R., Rudin D. (eds.). Springer Verlag, Berlin, pp. 127–142.
- Müller-Starck G., Baradat P.H., Bergmann F. 1992. Genetic variation within European tree species. In: *Population Genetics of Forest Trees*. Adams W.T., Strauss S.H., Copes D.L. (eds.). Kluwer Academic Publishers, Amsterdam, pp. 23–47.
- Pacalaj M., Longauer R., Krajmerová D., Gömöry D. 2002. Effect of site altitude on growth and survival of Norway spruce (*Picea abies* L.) provenances on the Slovak plots of IUFRO experiment 1972. *Journal of Forest Science* 48(1): 16–26.
- Skroppa T. 2003. *EUFORGEN Technical Guidelines for genetic conservation and use for Norway spruce (*Picea abies*)*. International Plant Genetic Resources Institute, Rome.
- Stefanović V. 1977. *Fitocenologija sa pregledom šumskih fitocenoza Jugoslavije*. Zavod za udžbenike, Sarajevo.
- Stefanović V., Beus V., Burlica Č., Dizdarević H., Vukorep I. 1983. *Ekološko-vegetacijska rejonizacija Bosne i Hercegovine*. Šumarski fakultet, Sarajevo, Posebna izdanja br. 17.
- Zupančič M. 1999. *Smrekovi gozdovi Slovenije*. Dela, 4. r., SAZU Ljubljana, 36.